

2139-11US FC

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NOT YET ASSIGNEDINTERNATIONAL APPLICATION NO.
PCT/CA97/00745INTERNATIONAL FILING DATE
15 October 1997PRIORITY DATE CLAIMED
---TITLE OF INVENTION
TRIVALENT THROMBIN INHIBITOR

APPLICANT(S) FOR DO/EO/US Yasuo KONISHI et al.

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2))
 - a. is transmitted herewith (required only if not transmitted by the International Bureau).
 - b. has been transmitted by the International Bureau.
 - c. is not required, as the application was filed in the United States Receiving Office (RO/US).
6. A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
 - a. are transmitted herewith (required only if not transmitted by the International Bureau).
 - b. have been transmitted by the International Bureau.
 - c. have not been made; however, the time limit for making such amendments has NOT expired.
 - d. have not been made and will not be made.
8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. A **FIRST** preliminary amendment.
- A **SECOND** or **SUBSEQUENT** preliminary amendment.
14. A substitute specification.
15. A change of power of attorney and/or address letter.
16. Other items or information:
 - Form PCT/IB/308
 - Copy of International Preliminary Examination Report

U.S. APPLICATION NO (if known, sec 37 CFR 1.5) 09/529232	INTERNATIONAL APPLICATION NO PCT/CA97/00745	ATTORNEY'S DOCKET NUMBER 2139-11US FC	
<p>17. <input type="checkbox"/> The following fees are submitted:</p> <p>BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :</p> <p>Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO \$970.00</p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO \$840.00</p> <p>International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO \$690.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) \$670.00</p> <p>International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) \$96.00</p>		CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$ 840.00	
<p>Surcharge of \$130.00 for furnishing the oath or declaration later than <input type="checkbox"/> 20 <input type="checkbox"/> 30 months from the earliest claimed priority date (37 CFR 1.492(e)).</p>		\$	
CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE
Total claims	14 - 20 =		X \$18.00
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MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ \$260.00
TOTAL OF ABOVE CALCULATIONS =		\$	
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<p>Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property</p>		+ \$ 40.00	
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<p>NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.</p>			
<p>SEND ALL CORRESPONDENCE TO:</p> <p>SWABEY OGILVY RENAULT 1981 McGill College Avenue Suite 1600 Montreal, Quebec H3A 2Y3 CANADA</p>			
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<p>NAME: _____</p>			
<p>37,037</p>			
<p>REGISTRATION NUMBER</p>			

TRIVALENT THROMBIN INHIBITORBACKGROUND OF THE INVENTION(a) Field of the Invention

5 The invention relates to a thrombin inhibitor, a pharmaceutical composition comprising same and a method for the treatment or prevention of vascular disease using the inhibitor of the present invention.

(b) Description of Prior Art

10 Thrombin plays a central role in the coagulation cascade of higher animals. The primary function of thrombin is to activate fibrinogen to fibrin and generate an insoluble fibrin clot. It also serves regulatory functions in coagulopathy by 15 activating several participating cofactors and proteases such as factor V, factor VIII, factor XIII and protein C. In a pathologic state, thrombin promotes coagulopathy, activates platelets and causes secretion of granular substances that exacerbate the 20 condition. Thrombin's interaction with endothelial cells, smooth muscle cells, fibroblasts, and monocytes/macrophages contribute further to the inflammatory process in thrombolytic events. Heart attack is one of the most important kind of 25 cardiovascular diseases. An acute blockage of a coronary artery by a thrombus causes a myocardial infarction. If a large artery, which nourishes a large part of the heart, is blocked, the attack is more likely to be fatal. In fact, 40% of death in North 30 America is attributes to cardiovascular diseases. The chances of recovery are good if the blockage occurs in one of the smaller coronary arteries. In its early stages, the condition may be alleviated with thrombolytic therapy. However, typical thrombolysis 35 with tissue plasminogen activator, urokinase or streptokinase is problematic. These enzymes activate

plasminogen to plasmin, which in turn lyses fibrin present in a coronary thrombus, thus restoring the blood flow to the heart muscle. Acute thrombotic reocclusion often occurs after initial successful 5 thrombolysis using these agents. Although the mechanism of reocclusion has not been clearly elucidated, thrombus-bound thrombin may contribute to this problem. In fact, thrombus-bound thrombin, which is still active, has been suggested to contribute to 10 rethrombosis after thrombolytic therapy (Agnelli, G., et al., *J. Thrombosis and Haemostasis* **66**, 592-597, 1991). Potent and specific agents that neutralize thrombus-bound thrombin would be desirable.

Thrombin is a member of the trypsin family of 15 serine proteases. In addition to the catalytic triad (Asp 102, His 57 and Ser 195) a feature common to the active site of all serine proteases, Asp 189 in the primary substrate binding site (S1) of the trypsin family plays an important role in the recognition and 20 binding of substrates and inhibitors.

Several approaches have been taken to design 25 anticoagulant agents. 1) Since one of the major component of thrombus is aggregated platelet-fibrinogen, drugs which prevent the aggregation between platelet and fibrinogen have been designed. A sequence of Arg-Gly-Asp in fibrinogen is responsible to interact with activated platelet so that many peptide or non-peptide based drugs which mimic the tripeptide structure have been developed. Antibodies which block 30 the platelet fibrinogen receptor, Gp IIb/IIIa, have also been developed. 2) Tissue factor pathway inhibitor, which inhibits tissue factor and factor VIIa complex, blocks the early stage of coagulation cascade. 3) Protein C is a natural anticoagulant and inactivates 35 factors Va and VIIIa. 4) Currently available drugs

which may not be optimized and use in a combination of the existing drugs has been studied. 5) Thrombin plays a central role in coagulation, thrombosis and platelet activation. The direct inhibition of thrombin activity 5 has advantages of independence to co-factors, efficacy to clot-bound thrombin, less patient-to-patient variability, and low risk of bleeding.

There is no satisfactory drug in the market. As a result, a natural anti-coagulant, heparin, which 10 has some side effects and low efficacy (but low cost), is still used in the hospital.

Heparin inhibits thrombin through a mechanism requiring a heparin-antithrombin III complex. Heparin is known to be poorly accessible to thrombus-bound 15 thrombin. Furthermore, heparin often causes bleeding when used therapeutically and is unable to prevent the occlusive complications in atherosclerotic vascular diseases or reocclusion following successful thrombolysis.

20 Another agent known to be effective for the inhibition of thrombus-bound thrombin is hirudin. Hirudin is produced by the salivary glands of the European medicinal leech *Hirudo medicinalis* and is a small protein of 65 amino acid residues. It has 25 several potential advantages over other antithrombotics. It is the most potent and specific thrombin inhibitor known having a K_i value of 2.2×10^{-14} M. Hirudin blocks the active site (AS) and the fibrinogen recognition exosite (FRE) of thrombin 30 simultaneously. Hirudin also inhibits thrombus-bound thrombin as well as circulating thrombin and it has a long half-life of 30-60 minutes when given intravenously or subcutaneously, depending on the species. Hirudin has very weak antigenicity, and it

has no reported acute side effect following intravenous or subcutaneous administration.

Synthetic thrombin inhibitors based on the hirudin sequence offer an advantage over native hirudin. They mimic the distinctive mechanism of hirudin and are more readily available through chemical synthesis. The crystal structure of the human α -thrombin/hirudin complex reveals that hirudin interacts with the enzyme through an active site inhibitor domain (hirudin¹⁻⁴⁸), a FRI inhibitor segment (hirudin⁵⁵⁻⁶⁵), and a linker segment (hirudin⁴⁹⁻⁵⁴) which connects these binding components.

The bulky active site inhibitor segment, hirudin¹⁻⁴⁸, is sufficiently large and serves to obstruct the enzyme surface. This action has been shown to be simulated when hirudin¹⁻⁴⁸ is replaced by a small active site inhibitor segment, (D-Phe)-Pro-Arg-Pro, with some loss in inhibitory potency (Maraganore, J.M., et al., *Biochemistry* **29**, 7095-7101, 1990; DiMaio, J., et al., *J.Biol.Chem.* **265**, 21698-21703, 1990; and Bourdon, P., et al., *FEBS Lett.* **294**, 163-166, 1991).

Investigators have focused on the use of (D-Phe)-Pro-Arg-Pro or its analog in the design of active site inhibitors. The crystal structure of (D-Phe)-Pro-Arg chloromethylketone (PPACK)-thrombin suggested that the (D-Phe)-Pro-Arg-Pro in bivalent inhibitors bind to the thrombin active site in a substrate binding mode, wherein Arg-X is the scissile peptide bond. The active site inhibitor segment, (D-Phe)-Pro-Arg-Pro, of the bivalent inhibitors is known to be hydrolyzed slowly by thrombin (DiMaio, J., et al., *Supra*; Witting, J.I., et al., *BioChem. J.* **287**, 663-664, 1992). The amino acids (D-Phe)-Pro-Arg comprised in the substrate type inhibitor (D-Phe)-Pro-

Arg-Pro bind to the S3, S2 and S1 subsites of thrombin, respectively.

Hirulog-8™ is a bivalent thrombin inhibitor composed of the substrate type inhibitor (D-Phe)-Pro-5 Arg-Pro, and the native sequence of the hirudin exosite segment 52-65 both linked through a suitable linker (Maraganore et al. US Patent No. 5,196,404). Since the structure of the active site inhibitor segment is very similar to the structure of PPAC, the interactions of 10 the substrate type active site inhibitor with thrombin is reasonably assumed to be the same as the interactions between the active site of PPAC and thrombin. In addition, it has been shown that the portion (D-Phe)-Pro-Arg-CO can be used in a bivalent 15 thrombin inhibitor (DiMaio et al. International publication WO 91/19734). The scissile position in a substrate is a position that is recognised by the enzyme and where the hydrolysis takes place. It is therefore advantageous to eliminate or to modify the 20 scissile position in order to give to more resistance to enzyme degradation. The synthesis of such inhibitors is difficult, cumbersome, uses dangerous chemicals and affords low yields of the desired compounds. There is therefore a need for other 25 thrombin inhibitors that would combine high inhibiting activity, enzyme resistance and affordable synthesis.

Besides substrate-type inhibitors, nonsubstrate type inhibitors could be designed to block the active site of thrombin without being cleaved. Examples of 30 these may be derived from arginine and benzamidine to give, for example, (2R,4R)-4-methyl-1-[N^a-(3-methyl-1,2,3,4-tetrahydro-8-quinolinesulphonyl)-L-arginyl]-2-piperidine carboxylic acid (MD-805), N^a-(4-toluene-sulphonyl)-D,L-amidinophenylalanyl-piperidine (TAPAP), 35 and Na-(2-naphthyl-sulphonyl-glycyl)-D-L,p-amidino-

phenylalanyl-piperidine (NAPAP). These active-site directed synthetic inhibitors have a short half-life of less than several minutes in the circulation. This activity is not of sufficient duration to be effective 5 against the continuous production of thrombin by the patient or against the effect of liberated thrombus bound-thrombin. The characteristic sequence of these compounds starting from the N-terminus is an aromatic group, arginyl or benzamidyl, and piperidine or its 10 analogs. In contrast to hirudin-based sequences, these moieties would be expected to occupy the S3, S1 and S2 subsites of the thrombin active site, respectively.

It has been previously reported that the combination of dansyl or dansyl analogues, arginine or 15 benzamidine, and pipecolic acid attaches to the thrombin active site. But it has been shown that such activity is weak and not pharmacologically useful (James C. Powers and Chih-Min Kam, *Thrombin: Structure and Function*, Chapter 4, (1992), Lawrence J. Berliner, 20 Plenum Press, New York).

A short sequence of a hirudin type inhibitor having non-substrate type active site inhibitor segment and the fibrinogen-recognition exosite inhibitor segment, has also been previously reported. (Tsuda, Y., 25 et al., *Biochemistry* 33: 14443-14451, 1994).

It would be highly desirable to be provided with a shortened thrombin inhibitor of the hirudin type. Such a shorter sequence would be easier to synthesize and cheaper to produce. It would have a 30 linear sequence less subject to enzymatic degradation and would be more stable when bound to thrombin.

SUMMARY OF THE INVENTION

One aim of the present invention is to provide 35 a new trivalent thrombin inhibitor which as a high

affinity for thrombin and which is more stable when such inhibitor is bound to thrombin.

In accordance with the present invention there is provided a new thrombin inhibitor of formula (I) or 5 a pharmaceutically acceptable salt thereof:

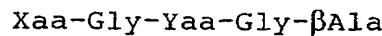


wherein

AS represents an S subsite blocking segment;

P represents a fibrinogen recognition exosite blocking 10 segment; and

Z represents a S' subsite blocking segment which links AS and P, said S' subsite blocking segment having the following sequence:



15 wherein Xaa is a residue selected from the group of residue consisting of glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, 20 L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, L-homo- 25 phenylalanine, D-homophenylalanine, L-histidine, D-histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine;

Yaa is selected from the group of residue consisting of 30 glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine,

D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, homophenylalanine, histidine, 5 L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine.

DETAILED DESCRIPTION OF THE INVENTION

10 Hirudin from medicinal leech is the most potent thrombin inhibitor. The high affinity of hirudin comes from the simultaneous binding to the active site and to the fibrinogen recognition exosite of thrombin. Synthetic thrombin inhibitors have been designed to 15 mimic the binding mode of hirudin and composed of the active site blocking segment, the fibrinogen recognition exosite blocking segment, and the linker connecting these blocking segments. Surprisingly, it has been found that two residues, identified as P1' and 20 P3', of the linker can form nonpolar interactions with thrombin. In accordance with the present invention, the linker, besides being a spacer, can be a binding segment to thrombin S' subsites. In this invention, the P1' and P3' residues were designed to optimize the 25 interactions with thrombin.

In accordance with the present invention, there is therefore provided a trivalent thrombin inhibitor comprising a S subsite blocking segment, which is connected to the S' subsite blocking segment, which is 30 connected to the fibrinogen recognition exosite blocking segment. In this invention, the design of the S' subsite blocking segment improved the affinity of the inhibitors by 250—300-fold which is significant and valuable commercially.

In accordance with one embodiment of the present invention, the trivalent thrombin inhibitors may be described by formula (I) which comprises an active site blocking segment (AS) linked to a S' sites 5 blocking segment (Z) which serves as a linker and a fibrinogen recognition exosite blocking segment (P) linked to that linker:



10

The AS blocking segment preferably has the following sequence:

Bbs-Arg-(D-Pip),

wherein Bbs and D-Pip represent 4-tert-15 butylbenzenesulfonyl and D-pipecolic acid, respectively.

The P segment preferably has the following sequences:

Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-20 Glu)-OH, SEQ ID NO:1
wherein Cha represent β -cyclohexyl-alanine.

The z segment preferably has the following sequence:

Xaa-Gly-Yaa-Gly- β Ala. SEQ ID NO:2

25 In accordance with a preferred embodiment of the present invention, when Xaa is a Glycine residue, Yaa is selected from the group of residue consisting of glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, 30 L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, 35 L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenyl-

glycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, homophenylalanine, histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine.

5 When Yaa is a glycine residue, Xaa is a residue selected from the group of residue consisting of glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, 10 L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, 15 L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, L-homophenylalanine, D-homophenylalanine, L-histidine, D-histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine.

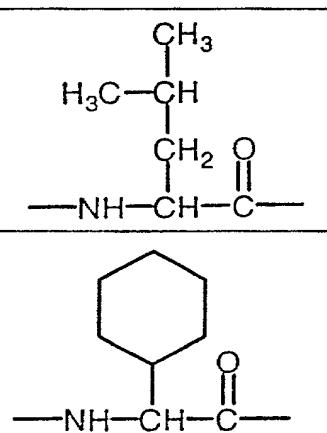
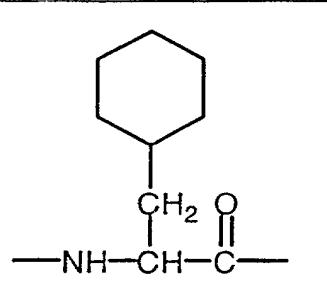
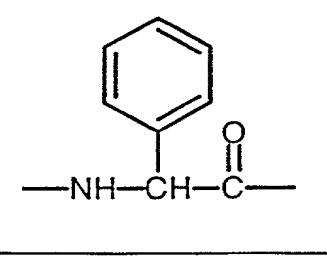
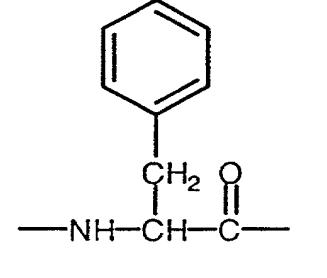
20 It should be noted that a person skilled in the art could substitute Gly, Gly and β -Ala in the Z segment with other amino acids, or could substitute both Xaa and Yaa simultaneously.

25 Table 1 below list the preferred Xaa and Yaa residues in accordance with the present invention, together with the Ki value, when available, of the inhibitor obtained. The following abbreviations have been used: α Aib, 2-aminoisobutyric acid; α Abu, α -aminobutyric acid; Bbs, 4-tert-butylbenzenesulfonyl; 30 Cha, β -cyclohexyl-alanine; Chg, cyclohexyl-glycine; Hph, homophenylalanine; Nva, norvaline; Nle, norleucine, Pen, Penicillamine; Phg, phenylglycine; Tbg, tert-butylglycine; and Thi, β -(2-thienyl)-alanine.

TABLE 1

Xaa or Yaa formula	P1' residue, Xaa Yaa = Gly	P3' residue, Yaa Xaa = Gly
$\text{---NH---CH}_2\text{---C---}$ O 	Xaa = Gly $K_i = 24 \pm 5 \text{ pM}$	Yaa = Gly $K_i = 24 \pm 5 \text{ pM}$
---NH---CH---C--- CH ₃ O 	Xaa = Ala $K_i = 1.2 \pm 0.4 \text{ pM}$ Xaa = D-Ala $K_i = 4.2 \pm 0.5 \text{ pM}$	Yaa = Ala $K_i = 8.7 \pm 0.2 \text{ pM}$ Yaa = D-Ala $K_i = 0.96 \pm 0.03 \text{ pM}$
---NH---C---C--- CH ₃ O CH ₃	Xaa = α Aib $K_i = 2.4 \pm 0.5 \text{ pM}$	Yaa = α Aib $K_i = 1.4 \pm 0.3 \text{ pM}$
---NH---CH---C--- CH ₃ CH ₂ O 	Xaa = α Abu $K_i = 0.63 \pm 0.05 \text{ pM}$ Xaa = D- α Abu $K_i = 4.25 \pm 0.4 \text{ pM}$	Yaa = α Abu $K_i = 7.4 \pm 0.3 \text{ pM}$ Yaa = D- α Abu $K_i = 0.77 \pm 0.03 \text{ pM}$
---NH---CH---C--- CH ₃ CH ₂ CH ₂ O 	Xaa = Nva $K_i = 0.24 \pm 0.05 \text{ pM}$ Xaa = D-Nva $K_i = 5.1 \pm 0.4 \text{ pM}$	Yaa = Nva $K_i = 9.2 \pm 0.4 \text{ pM}$ Yaa = D-Nva $K_i = 0.88 \pm 0.04 \text{ pM}$
---NH---CH---C--- CH ₃ CH ₂ CH ₂ CH ₂ O 	Xaa = Nle $K_i = 0.082 \pm 0.006 \text{ pM}$ Xaa = D-Nle $K_i = 5.3 \pm 0.3 \text{ pM}$	Yaa = Nle $K_i = 8.9 \pm 0.5 \text{ pM}$ Yaa = D-Nle $K_i = 0.68 \pm 0.04 \text{ pM}$

$ \begin{array}{c} \text{SH} \\ \\ \text{CH}_2 \text{ O} \\ \\ \text{---NH---CH---C---} \end{array} $	Xaa = Cys $K_i = 1.2 \pm 0.3 \text{ pM}$	Yaa = Cys $K_i = 8.9 \pm 0.5 \text{ pM}$
$ \begin{array}{c} \text{SH} \\ \\ \text{H}_3\text{C---C---CH}_3 \\ \\ \text{---NH---CH---C---} \\ \\ \text{O} \end{array} $	Xaa = Pen $K_i = 1.5 \pm 0.4 \text{ pM}$ Xaa = D-Pen $K_i = 6.8 \pm 0.5 \text{ pM}$	Yaa = Pen $K_i = 5.6 \pm 0.5 \text{ pM}$ Yaa = D-Pen $K_i = 1.5 \pm 0.5 \text{ pM}$
$ \begin{array}{c} \text{CH}_3 \\ \\ \text{S} \\ \\ \text{CH}_2 \\ \\ \text{CH}_2 \text{ O} \\ \\ \text{---NH---CH---C---} \end{array} $	Xaa = Met $K_i = 0.11 \pm 0.03 \text{ pM}$ Xaa = D-Met $K_i = 4.8 \pm 0.3 \text{ pM}$	Yaa = Met $K_i = 10.4 \pm 0.5 \text{ pM}$ Yaa = D-Met $K_i = 1.5 \pm 0.5 \text{ pM}$
$ \begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C---CH} \\ \\ \text{O} \\ \text{---NH---CH---C---} \end{array} $	Xaa = Val $K_i = 0.84 \pm 0.05 \text{ pM}$ Xaa = D-Val $K_i = 3.7 \pm 0.4 \text{ pM}$	Yaa = Val $K_i = 1.2 \pm 0.5 \text{ pM}$ Yaa = D-Val $K_i = 0.62 \pm 0.02 \text{ pM}$
$ \begin{array}{c} \text{CH}_3 \\ \\ \text{H}_3\text{C---C---CH}_3 \\ \\ \text{---NH---CH---C---} \\ \\ \text{O} \end{array} $	Xaa = Tbg $K_i = 1.1 \pm 0.3 \text{ pM}$ Xaa = D-Tbg $K_i = 5.8 \pm 0.4 \text{ pM}$	Yaa = Tbg $K_i = 4.3 \pm 0.5 \text{ pM}$ Yaa = D-Tbg $K_i = 0.44 \pm 0.04 \text{ pM}$
$ \begin{array}{c} \text{CH}_3 \\ \\ \text{CH}_2 \\ \\ \text{H}_3\text{C---CH} \\ \\ \text{O} \\ \text{---NH---CH---C---} \end{array} $	Xaa = Ile $K_i = 0.14 \pm 0.04 \text{ pM}$ Xaa = D-Ile $K_i = 4.3 \pm 0.3 \text{ pM}$	Yaa = Ile $K_i = 1.9 \pm 0.3 \text{ pM}$ Yaa = D-Ile $K_i = 0.35 \pm 0.5 \text{ pM}$

	Xaa = Leu $K_i = 0.12 \pm 0.04 \text{ pM}$ Xaa = D-Leu $K_i = 2.6 \pm 0.5 \text{ pM}$	Yaa = Leu $K_i = 2.2 \pm 0.5 \text{ pM}$ Yaa = D-Leu $K_i = 0.62 \pm 0.03 \text{ pM}$
	Xaa = Chg $K_i = 0.35 \pm 0.5 \text{ pM}$	Yaa = Chg $K_i = 3.2 \pm 0.4 \text{ pM}$
	Xaa = Cha $K_i = 0.12 \pm 0.04 \text{ pM}$ Xaa = D-Cha $K_i = 7.2 \pm 0.3 \text{ pM}$	Yaa = Cha $K_i = 9.6 \pm 0.5 \text{ pM}$ Yaa = D-Cha $K_i = 1.5 \pm 0.5 \text{ pM}$
	Xaa = Phg $K_i = 3.1 \pm 0.4 \text{ pM}$ Xaa = D-Phg $K_i = 7.8 \pm 0.5 \text{ pM}$	Yaa = Phg $K_i = 2.8 \pm 0.4 \text{ pM}$ Yaa = D-Phg $K_i = 0.095 \pm 0.006 \text{ pM}$
	Xaa = Phe $K_i = 0.51 \pm 0.05 \text{ pM}$ Xaa = D-Phe $K_i = 3.4 \pm 0.3 \text{ pM}$	Yaa = Phe $K_i = 4.5 \pm 0.4 \text{ pM}$ Yaa = D-Phe $K_i = 0.13 \pm 0.05 \text{ pM}$

	Xaa = Hph $K_i = 0.18 \pm 0.05 \text{ pM}$ Xaa = D-Hph $K_i = 2.8 \pm 0.5 \text{ pM}$	Yaa = Hph $K_i = 7.2 \pm 0.3 \text{ pM}$ Yaa = D-Hph $K_i = 0.78 \pm 0.05 \text{ pM}$
	Xaa = His $K_i = 0.91 \pm 0.04 \text{ pM}$ Xaa = D-His $K_i = 2.1 \pm 0.3 \text{ pM}$	Yaa = His $K_i = 6.2 \pm 0.5 \text{ pM}$ Yaa = D-His $K_i = 1.4 \pm 0.4 \text{ pM}$
	Xaa = Trp $K_i = 630 \pm 30 \text{ pM}$ Xaa = D-Trp $K_i = 820 \pm 50 \text{ pM}$	Yaa = Trp $K_i = 9.8 \pm 0.4 \text{ pM}$ Yaa = D-Trp $K_i = 2.2 \pm 0.5 \text{ pM}$
	Xaa = Thi $K_i = 0.051 \pm 0.004 \text{ pM}$ Xaa = D-Thi $K_i = 2.8 \pm 0.4 \text{ pM}$	Yaa = Thi $K_i = 5.1 \pm 0.5 \text{ pM}$ Yaa = D-Thi $K_i = 1.2 \pm 0.4 \text{ pM}$

The preferred inhibitors having a K_i value smaller than 1 pM in accordance with the present invention are:

1) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Ala)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

2) Bbs-Arg-(D-Pip)- α Abu-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

3) Bbs-Arg-(D-Pip)-Gly-Gly-(D- α Abu)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

10 4) Bbs-Arg-(D-Pip)-Nva-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

5) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Nva)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
15 (SEQ ID NO:3)

6) Bbs-Arg-(D-Pip)-Nle-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

7) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Nle)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
20 (SEQ ID NO:3)

8) Bbs-Arg-(D-Pip)-Met-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

25 9) Bbs-Arg-(D-Pip)-Val-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

10) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Val)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

11) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Tbg)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
5
(SEQ ID NO:3)

12) Bbs-Arg-(D-Pip)-Ile-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

10 13) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Ile)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

14) Bbs-Arg-(D-Pip)-Leu-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
15
(SEQ ID NO:3)

15) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Leu)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

16) Bbs-Arg-(D-Pip)-Chg-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
20
(SEQ ID NO:3)

17) Bbs-Arg-(D-Pip)-Cha-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

25 18) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Phg)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

19) Bbs-Arg-(D-Pip)-Phe-Gly-Gly-Gly- β Ala-Asp-Tyr-
Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

20) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Phe)-Gly- β Ala-Asp-
5 Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

21) Bbs-Arg-(D-Pip)-Hph-Gly-Gly-Gly- β Ala-Asp-Tyr-
Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

10 22) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Hph)-Gly- β Ala-Asp-
Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

23) Bbs-Arg-(D-Pip)-His-Gly-Gly-Gly- β Ala-Asp-Tyr-
Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;

15 (SEQ ID NO:3)

and

24) Bbs-Arg-(D-Pip)-Thi-Gly-Gly-Gly- β Ala-Asp-Tyr-
Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH
(SEQ ID NO:3).

20 The more preferred inhibitors having a K_i value
smaller than 0.1 pM in accordance with the present
invention are:

1) Bbs-Arg-(D-Pip)-Nle-Gly-Gly-Gly- β Ala-Asp-Tyr-
Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
25 (SEQ ID NO:3)

2) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Phg)-Gly- β Ala-Asp-
Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

and

3) Bbs-Arg-(D-Pip)-Thi-Gly-Gly-Gly- β Ala-Asp-Tyr-
Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH
(SEQ ID NO:3).

5 It should be noted that a person skilled in the art could substitute suitable active site blocking segment, S' subsite blocking segment and fibrinogen recognition exosite blocking segment, and synthesize variants of such active trivalent hirudin-like
10 inhibitors.

The inhibitors of the present invention have a higher affinity than the inhibitors of the prior art. This therefore represents a concrete benefit in the field of thrombin inhibitors.

15 While it may be possible that, for use in therapy, a compound of the invention may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

20 It will be appreciated by those skilled in the art that the compounds of formula (I) may be modified to provide pharmaceutically acceptable salts thereof which are included within the scope of the invention.

25 Pharmaceutically acceptable salts of the compounds of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, 30 lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic, 35 may be useful as intermediates in obtaining the

compounds of the invention and their pharmaceutically acceptable acid addition salts.

The invention thus further provides a pharmaceutical formulation comprising a compound of formula (I) and pharmaceutically acceptable acid addition salt thereof together with one or more pharmaceutically acceptable carriers therefor and, optionally, other therapeutic and/or prophylactic ingredients. The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not deleterious to the recipient thereof.

In a further embodiment of the present invention is provided the use of a compounds of formula (I) or a pharmaceutically acceptable salt in the manufacture of a medicament for the treatment of vascular disease in a mammal including human.

In an alternative aspect of the present invention is provided a method for the treatment of vascular disease for the treatment of a mammal, including human comprising the administration of an effective amount of a compound of formula (I).

It will be appreciated by people skilled in the art that treatment extends to prophylaxis as well to the treatment of established vascular disease.

The compounds of the present invention are useful in combinations, formulations and methods for the treatment and prophylaxis of vascular diseases. These diseases include myocardial infarction, stroke, pulmonary embolism, deep vein thrombosis, peripheral arterial occlusion, restenosis following arterial injury or invasive cardiological procedures, acute or chronic atherosclerosis, edema and inflammation, cancer and metastasis.

The term "combination" as used herein, includes a single dosage form containing at least one compound of this invention and at least one thrombolytic agent, a multiple dosage form, wherein the thrombin inhibitor 5 and the thrombolytic agent are administered separately, but concurrently, or a multiple dosage form wherein the two components are administered separately, but sequentially. In sequential administration, the thrombin inhibitor may be given to the patient during 10 the time period ranging from about 5 hours prior to about 5 hours after administration of the thrombolytic agent. Preferably, the thrombin inhibitor is administered to the patient during the period ranging from 2 hours prior to 2 hours following administration 15 of the thrombolytic agent.

In these combinations, the thrombin inhibitor and the thrombolytic agent work in a complementary fashion to dissolve blood clots, resulting in decreased reperfusion times and increased reocclusion times in 20 patients treated with them. Specifically, the thrombolytic agent dissolves the clot, while the thrombin inhibitor prevents newly exposed, clot-entrapped or clot-bound thrombin from regenerating the clot. The use of the thrombin inhibitor in the 25 formulations of this invention advantageously allows the administration of a thrombolytic reagent in dosages previously considered too low to result in thrombolytic effects if given alone. This avoids some of the undesirable side effects associated with the use of 30 thrombolytic agents, such as bleeding complications.

Thrombolytic agents which may be employed in the combinations of the present invention are those known in the art. Such agents include, but are not limited to, tissue plasminogen activator purified from 35 natural sources, recombinant tissue plasminogen

activator, streptokinase, urokinase, purokinase, anisolated streptokinase plasminogen activator complex (ASPAC), animal salivary gland plasminogen activators and known, biologically active derivatives of any of 5 the above.

Various dosage forms may be employed to administer the formulations and combinations of this invention. These include, but are not limited to, parenteral administration, oral administration and 10 topical application. The formulations and combinations of this invention may be administered to the patient in any pharmaceutically acceptable dosage form, including those which may be administered to a patient intravenously as bolus or by continued infusion, 15 intramuscularly -- including paravertebrally and periarticularly -- subcutaneously, intracutaneously, intra-articularly, intrasynovially, intrathecally, intra-lesionally, periostally or by oral, nasal, or topical routes. Such compositions and combinations are 20 preferably adapted for topical, nasal, oral and parenteral administration, but, most preferably, are formulated for parenteral administration.

Parenteral compositions are most preferably administered intravenously either in a bolus form or as 25 a constant infusion. For parenteral administration, fluid unit dose forms are prepared which contain the compounds of the present invention and a sterile vehicle. The compounds of this invention may be either suspended or dissolved, depending on the nature of the 30 vehicle and the nature of the particular compounds of this invention. Parenteral compositions are normally prepared by dissolving the compounds of this invention in a vehicle, optionally together with other components, and filter sterilizing before filling into 35 a suitable vial or ampule and sealing. Preferably,

adjuvants such as a local anesthetic, preservatives and buffering agents are also dissolved in the vehicle. The composition may then be frozen and lyophilized to enhance stability.

5 Parenteral suspensions are prepared in substantially the same manner, except that the active component is suspended rather than dissolved in the vehicle. Sterilization of the compositions is preferably achieved by exposure to ethylene oxide
10 before suspension in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of its components.

Tablets and capsules for oral administration
15 may contain conventional excipients, such as binding agents, fillers, diluents, tabletting agents, lubricants, disintegrants, and wetting agents. The tablet may be coated according to methods well known in the art. Suitable fillers which may be employed
20 include cellulose, mannitol, lactose and other similar agents. Suitable disintegrants include, but are not limited to, starch, polyvinylpyrrolidone and starch derivatives, such as sodium starch glycolate. Suitable lubricants include, for example, magnesium stearate.
25 Suitable wetting agents include sodium lauryl sulfate.

Oral liquid preparations may be in the form of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for reconstitution with water or another suitable vehicle before use. Such liquid preparations may contain conventional additives. These include suspending agents, such as sorbitol, syrup, methyl cellulose, gelatin, hydroxyethylcellulose, carboxymethylcellulose, aluminum stearate gel or 35 hydrogenated edible fats, emulsifying agents which

include lecithin, sorbitan monooleate, polyethylene glycols, or acacia, non-aqueous vehicles, such as almond oil, fractionated coconut oil, and oily esters, and preservatives, such as methyl or propyl p-5 hydroxybenzoate or sorbic acid.

Formulations for topical administration may, for example, be in aqueous jelly, oily suspension or emulsified ointment form.

The dosage and dose rate of the compounds of 10 this invention will depend on a variety of factors, such as the weight of the patient, the specific pharmaceutical composition used, the object of the treatment, i.e., therapy or prophylaxis, the nature of the thrombotic disease to be treated, and the judgment 15 of the treating physician.

According to the present invention, a preferred pharmaceutically effective daily dose of the compounds of this invention is between about 1 $\mu\text{g}/\text{kg}$ body weight of the patient to be treated ("body weight") and about 20 5 mg/kg body weight. In combinations containing a thrombolytic agent, a pharmaceutically effective daily dose of the thrombolytic is between about 10% and 80% of the conventional dosage range. The "conventional dosage range" of a thrombolytic agent is the daily 25 dosage used when that agent is employed in a monotherapy [physician's Desk Reference 1989, 43rd Edition, Edward R. Barnhart, publisher]. That conventional dosage range will, of course, vary depending on the thrombolytic agent employed. Examples 30 of conventional dosage ranges are as follows: urokinase - 500,000 to 6,250,000 units/patient, streptokinase - 140,000 to 2,500,000 units/patient, tPA - 0.5 to 5.0 mg/kg body weight, ASPAC - 0.1 to 10 units/kg body weight.

Most preferably, the therapeutic and prophylactic compositions of the present invention comprise a dosage of between about 10 $\mu\text{g}/\text{kg}$ body weight and about 500 $\mu\text{g}/\text{kg}$ body weight of the compounds of this invention. Most preferred combinations comprise the same amount of the compounds of this invention and between about 10% and about 70% of the conventional dosage range of a thrombolytic agent. It should also be understood that a daily pharmaceutically effective dose of either the compounds of this invention or the thrombolytic agent present in combinations of the invention, may be less than or greater than the specific ranges cited above.

Once improvement in the patient's condition has occurred, a maintenance dose of a combination or composition of this invention is administered, if necessary. Subsequently, the dosage or the frequency of administration, or both, may be reduced, as a function of the symptoms, to a level at which the improved condition is retained. When the symptoms have been alleviated to the desired level, treatment should cease. Patients may, however, require intermittent treatment upon any recurrence of disease symptoms.

According to an alternate embodiment of this invention, compounds may be used in compositions and methods for coating the surfaces of invasive devices, resulting in a lower risk of clot formation or platelet activation in patients receiving such devices. Surfaces that may be coated with the compositions of this invention include, for example, prostheses, artificial valves, vascular grafts, stents and catheters. Methods and compositions for coating these devices are known to those of skill in the art. These include chemical cross-linking or physical adsorption of the compounds of this invention-containing

compositions to the surfaces of the devices. According to a further embodiment of the present invention, compounds may be used for ex vivo thrombus imaging in a patient. In this embodiment, the compounds of this 5 invention are labeled with a radioisotope. The choice of radioisotope is based upon a number of well-known factors, for example, toxicity, biological half-life and detectability. Preferred radioisotopes include, but are not limited to ^{125}I , ^{123}I and ^{111}I . Techniques 10 for labeling the compounds of this invention are well known in the art. Most preferably, the radioisotope is ^{123}I and the labeling is achieved using ^{123}I -Bolton-Hunter Reagent. The labeled thrombin inhibitor is administered to a patient and allowed to bind to the 15 thrombin contained in a clot. The clot is then observed by utilizing well-known detecting means, such as a camera capable of detecting radioactivity coupled to a computer imaging system. This technique also yields images of platelet-bound thrombin and 20 meizothrombin.

This invention also relates to compositions containing the compounds of this invention and methods for using such compositions in the treatment of tumor metastases. The efficacy of the compounds of this 25 invention for the treatment of tumor metastases is manifested by the inhibition inhibitors to inhibit thrombin-induced endothelial cell activation. This inhibition includes the repression of platelet activation factor (PAF) synthesis by endothelial cells. 30 These compositions and methods have important applications in the treatment of diseases characterized by thrombin-induced inflammation and edema, which is thought to be mediated by PAF. Such diseases include, but are not limited to, adult respiratory distress 35 syndrome, septic shock, septicemia and reperfusion

damage. Early stages of septic shock include discrete, acute inflammatory and coagulopathic responses. It has previously been shown that injection of baboons with a lethal dose of live *E. coli* leads to marked declines in 5 neutrophil count, blood pressure and hematocrit. Changes in blood pressure and hematocrit are due in part to the generation of a disseminated intravascular coagulopathy (DIC) and have been shown to parallel consumption of fibrinogen (F. B. Taylor et al., *J. 10 Clin. Invest.*, **79**, pp. 918-25, 1987). Neutropenia is due to the severe inflammatory response caused by septic shock which results in marked increases in tumor necrosis factor levels. The compounds of this 15 invention may be utilized in compositions and methods for treating or preventing DIC in septicemia and other diseases.

This invention also relates to the use of the above-described compounds, or compositions comprising them, as anticoagulants for extracorporeal blood. As 20 used herein, the term "extracorporeal blood" includes blood removed in line from a patient, subjected to extracorporeal treatment, and then returned to the patient in such processes as dialysis procedures, blood filtration, or blood bypass during surgery. The term 25 also includes blood products which are stored extracorporeally for eventual administration to a patient and blood collected from a patient to be used for various assays. Such products include whole blood, plasma, or any blood fraction in which inhibition of 30 coagulation is desired.

The amount or concentration of compounds of this invention in these types of compositions is based on the volume of blood to be treated or, more preferably, its thrombin content. Preferably, an 35 effective amount of a compounds of this invention of

this invention for preventing coagulation in extracorporeal blood is from about 1 μ g/60 ml of extracorporeal blood to about 5 mg/60 ml of extracorporeal blood.

5 The compounds of this invention may also be used to inhibit clot-bound thrombin, which is believed to contribute to clot accretion. This is particularly important because commonly used anti-thrombin agents, such as heparin and low molecular weight heparin, are
10 ineffective against clot-bound thrombin. Finally, the compounds of this invention may be employed in compositions and methods for treating neurodegenerative diseases. Thrombin is known to cause neurite retraction, a process suggestive of the rounding in
15 shape changes of brain cells and implicated in neurodegenerative diseases, such as Alzheimer's disease and Parkinson's disease.

The present invention will be more readily understood by referring to the following examples which
20 are given to illustrate the invention rather than to limit its scope.

EXAMPLE I EXPERIMENTAL PROCEDURES

25 Materials

Human α -thrombin (3000 NIH units/mg), Tos-Gly-Pro-Arg-AMC·HCl salt, poly(ethylene glycol) 8000, and Tris were purchased from Sigma. All Fmoc-amino acids, and all other amino acid derivatives for peptide
30 synthesis were purchased from Advanced ChemTech, Bachem Bioscience Inc. and Calbiochem-Novabiochem. Fmoc-D-Glu(OtBu)-Wang resin (0.59 mmol/g) was purchased from Calbiochem-Novabiochem. The solvents for peptide synthesis were obtained from B&J Chemicals and Applied

Biosystems Inc. Trifluoroacetic acid was purchased from Halocarbon Products Co.

Peptide synthesis

The peptides were synthesized by the solid-phase method on a 396 Multiple Peptide Synthesizer (Advanced ChemTech) using a conventional Fmoc procedure. Peptides were cleaved from the resin using Reagent K (TFA 82.5%/water 5%/phenol 5%/thioanisole 5%/ethanedithiol 2.5%; 25 mL/g of peptide-resin) for 2-4 hours at room temperature. After precipitation with diethyl ether, peptides were filtered, dissolved in 50% acetic acid, and lyophilized. The peptides were then purified by a preparative HPLC (Vydac C₄ column, 4.6 X 25 cm) using a linear gradient of 20 to 50% acetonitrile in 0.1% TFA (0.5%/min gradient, 33 mL/min flow rate). The final products were lyophilized with 98% or higher purity estimated by an analytical HPLC (Vydac C₁₈, 0.46 X 25 cm column, 10-60% acetonitrile in 0.1% TFA, 1.0%/min gradient, 1.0 mL/min flow rate). The elution profile was monitored by an absorbance at 210 and 254 nm for the analytical HPLC, and 220 nm for the preparative HPLC. The peptides were identified with a Beckmann model 6300 amino acid analyzer and a SCIEX API III mass spectrometer. Amino acid analysis was used for peptide content determination. All peptides used in this article have correct amino acid composition and molecular mass.

Amidolytic assays

The inhibition of amidolytic activity of human α -thrombin was measured fluorometrically using Tos-Gly-Pro-Arg-AMC as a fluorogenic substrate in 50 mM Tris-HCl buffer (pH 7.80 at 25°C) containing 0.1 M NaCl and 0.1% poly(ethylene glycol) 8000 at room temperature (Szewczuk Z. et al. *Biochemistry* 31: 9132-9140, 1992). The final concentration of the inhibitors, the

substrate and human α -thrombin were 0.5-1000-fold of K_i , $(1-8) \times 10^{-6}$ M, and 3.0×10^{-11} M, respectively, if $K_i > 10^{-10}$ M; 10-100-fold of K_i , $(5-40) \times 10^{-6}$ M, and 3.0×10^{-11} M, respectively, if 10^{-10} M $> K_i > 10^{-11}$ M; and 5 $(2-60) \times 10^{-10}$ M, $(5-40) \times 10^{-6}$ M, and 3.0×10^{-11} M, respectively, if $K_i < 10^{-11}$ M. The hydrolysis of the substrate by thrombin was monitored on a Perkin Elmer LS50B luminescence spectrometer ($\lambda_{ex}=383$ nm; $\lambda_{em}=455$ nm) or on a Hitachi F2000 fluorescence 10 spectrophotometer ($\lambda_{ex}=383$ nm; $\lambda_{em}=455$ nm), and the fluorescent intensity was calibrated using AMC. The substrate and an inhibitor were pre-mixed at appropriate concentrations (the solution volume was adjusted to 2.99 mL) before adding 10 μ L of human 15 α -thrombin (9×10^{-9} M). The reaction reached a steady state within 3 min after the hydrolysis started. The steady-state velocity was then measured for a few minutes. The kinetic data of the steady-state velocity at various concentrations of the substrate and the 20 inhibitors of the competitive inhibition were analyzed using the methods described by Segal (Enzyme Kinetics: Behavior and Analysis of Rapid Equilibrium and Steady-State Enzyme Systems pp 100-160, John Wiley & Sons, 1975) and Szewczuk et al. (Biochemistry, 32: 3396-3404, 25 1993). A nonlinear regression program (Microsoft Excel) was used to estimate the kinetic parameters (K_m , V_{max} , and K_i). Some inhibitors with high affinity to thrombin showed a biphasic progress curves of the fluorescence change by time, which is typical phenomena 30 of slow-tight binding inhibition (Morrison & Walsh, Adv. in Enzymol. 61: 201-301, 1988). The progress curves of the slow-tight binding inhibition were analyzed by the methods described by Stone & Hofsteenge (Biochemistry 25: 4622-4628, 1986).

While the invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modifications and this application is intended to cover any 5 variations, uses, or adaptations of the invention following, in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as 10 may be applied to the essential features hereinbefore set forth, and as follows in the scope of the appended claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: National Research Council of Canada
- (B) STREET: 1200 Montreal Road, Building M-58, Rm EG-12
- (C) CITY: Ottawa
- (D) STATE: Ontario
- (E) COUNTRY: Canada
- (F) POSTAL CODE (ZIP): K1A 0R6
- (G) TELEPHONE: 613-993-3899
- (H) TELEFAX: 613-952-6082

(ii) TITLE OF INVENTION: TRIVALENT THROMBIN INHIBITOR

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:10
- (D) OTHER INFORMATION:/Xaa= "beta-cyclohexyl-alanine"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:11
- (D) OTHER INFORMATION:/Xaa= "D-glutamic acid"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asp Tyr Glu Pro Ile Pro Glu Glu Ala Xaa Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 5 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

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(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:1
- (D) OTHER INFORMATION:/Xaa= "glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, L-homophenylalanine, D-homophenylalanine, L-histidine, D-histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:3
- (D) OTHER INFORMATION:/Xaa= "glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, homophenylalanine, histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine"

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) LOCATION:5
- (D) OTHER INFORMATION:/Xaa= "bAla"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Xaa Gly Xaa Gly Xaa
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 19 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

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(A) NAME/KEY: Modified-site
(B) LOCATION:1
(D) OTHER INFORMATION:/Xaa= "4-tert-butylbezenesulfonyl"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION:3
(D) OTHER INFORMATION:/Xaa= "D-pipecolic acid"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION:4
(D) OTHER INFORMATION:/Xaa= "glycine, L-alanine, D-alanine,
2-aminoisobutyric acid, L- α -aminobutyric acid,
D- α -aminobutyric acid, L-norvaline, D-norvaline,
L-norleucine, D-norleucine, L-cysteine,
L-penicillamine, D-penicillamine, L-methionine,
D-methionine, L-valine, D-valine, L-tert-butylglycine,
D-tert-butylglycine, L-isoleucine, D-isoleucine,
L-leucine, D-leucine, cyclohexylglycine,
L- β -cyclohexylalanine, D- β -cyclohexylalanine,
L-phenylglycine, D-phenylglycine, L-phenylalanine,
D-phenylalanine, L-homophenylalanine,
D-homophenylalanine, L-histidine, D-histidine,
L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine,
and D- β -(2-thienyl)-alanine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION:6
(D) OTHER INFORMATION:/Xaa= "glycine, L-alanine,
D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric
acid, D- α -aminobutyric acid, L-norvaline, D-norvaline,
L-norleucine, D-norleucine, L-cysteine,
L-penicillamine, D-penicillamine, L-methionine,
D-methionine, L-valine, D-valine, L-tert-butylglycine,
D-tert-butylglycine, L-isoleucine, D-isoleucine,
L-leucine, D-leucine, cyclohexylglycine,
L- β -cyclohexylalanine, D- β -cyclohexylalanine,
L-phenylglycine, D-phenylglycine, L-phenylalanine,
D-phenylalanine, homophenylalanine, histidine,
L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine,
and D- β -(2-thienyl)-alanine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION:8
(D) OTHER INFORMATION:/Xaa= "bAla"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION:18
(D) OTHER INFORMATION:/Xaa= "beta-cyclohexyl-alanine"

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) LOCATION:19
(D) OTHER INFORMATION:/Xaa= "D-glutamic acid"

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Xaa Arg Xaa Xaa Gly Xaa Gly Xaa Asp Tyr Glu Pro Ile Pro Glu Glu
1 5 10 15

Ala Xaa Xaa

WHAT IS CLAIMED IS:

1. A thrombin inhibitor of formula (I) or a pharmaceutically acceptable salt thereof:

AS-Z-P (I)

wherein

AS represents an S subsite blocking segment;

P represents a fibrinogen recognition exosite blocking segment; and

Z represents a S' subsite blocking segment which links AS and P, said S' subsite blocking segment having the following sequence:

Xaa-Gly-Yaa-Gly- β Ala

wherein Xaa is a residue selected from the group of residue consisting of glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclohexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, L-homo-phenylalanine, D-homophenylalanine, L-histidine, D-histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine;

Yaa is selected from the group of residue consisting of glycine, L-alanine, D-alanine, 2-aminoisobutyric acid, L- α -aminobutyric acid, D- α -aminobutyric acid, L-norvaline, D-norvaline, L-norleucine, D-norleucine, L-cysteine, L-penicillamine, D-penicillamine, L-methionine, D-methionine, L-valine, D-valine, L-tert-butylglycine, D-tert-butylglycine, L-isoleucine, D-isoleucine, L-leucine, D-leucine, cyclo-

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hexylglycine, L- β -cyclohexylalanine, D- β -cyclohexylalanine, L-phenylglycine, D-phenylglycine, L-phenylalanine, D-phenylalanine, homophenylalanine, histidine, L-tryptophan, D-tryptophan, L- β -(2-thienyl)-alanine, and D- β -(2-thienyl)-alanine.

2. The thrombin inhibitor of claim 1, wherein A_S has the following sequence:

Bbs-Arg-(D-Pip),
wherein Bbs and D-Pip represent 4-tert-butylbenzenesulfonyl and D-pipecolic acid, respectively.

3. The thrombin inhibitor of claim 1, wherein P has the following sequence:

Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH,
wherein Cha represent β -cyclohexyl-alanine.

4. The thrombin inhibitor of claim 2, wherein P has the following sequence:

Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH,
wherein Cha represent β -cyclohexyl-alanine.

5. The compound of claim 1, wherein said compound is selected from the group consisting of:

- 1) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Ala)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)
- 2) Bbs-Arg-(D-Pip)- α Abu-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

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3) Bbs-Arg-(D-Pip)-Gly-Gly-(D- α Abu)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

4) Bbs-Arg-(D-Pip)-Nva-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

5) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Nva)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

6) Bbs-Arg-(D-Pip)-Nle-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

7) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Nle)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

8) Bbs-Arg-(D-Pip)-Met-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

9) Bbs-Arg-(D-Pip)-Val-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

10) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Val)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

11) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Tbg)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

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12) Bbs-Arg-(D-Pip)-Ile-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

13) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Ile)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

14) Bbs-Arg-(D-Pip)-Leu-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

15) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Leu)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

16) Bbs-Arg-(D-Pip)-Chg-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

17) Bbs-Arg-(D-Pip)-Cha-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

18) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Phg)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

19) Bbs-Arg-(D-Pip)-Phe-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

20) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Phe)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

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21) Bbs-Arg-(D-Pip)-Hph-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

22) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Hph)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

23) Bbs-Arg-(D-Pip)-His-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

and

24) Bbs-Arg-(D-Pip)-Thi-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH
(SEQ ID NO:3).

6. The compound of claim 1, wherein said compound is selected from the group consisting of:

1) Bbs-Arg-(D-Pip)-Nle-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

2) Bbs-Arg-(D-Pip)-Gly-Gly-(D-Phg)-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH;
(SEQ ID NO:3)

and

3) Bbs-Arg-(D-Pip)-Thi-Gly-Gly-Gly- β Ala-Asp-Tyr-Glu-Pro-Ile-Pro-Glu-Glu-Ala-Cha-(D-Glu)-OH
(SEQ ID NO:3).

7. Use of a compound as defined in claim 1 in the manufacture of a medicament for the treatment of vascular diseases in a mammal.

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8. The use of claim 7, wherein said mammal is a human.

9. A pharmaceutical composition for treating or preventing vascular disease, said composition comprising a therapeutically effective amount of a compound as defined in claim 1, and a pharmaceutically acceptable carrier.

10. A pharmaceutically acceptable combination for treating or preventing vascular disease in a mammal, comprising a compound as defined in claim 1, a thrombolytic agent and a pharmaceutically acceptable carrier.

11. The combination according to claim 10, wherein said thrombolytic agent is tissue plasminogen activator.

12. The combination according to claim 10, wherein said mammal is a human.

13. A method for the treatment or prevention of vascular diseases of a mammal comprising the administration of an effective amount of a composition according to claim 10.

14. The method according to claim 13, wherein said mammal is a human.

COMBINED DECLARATION FOR UTILITY OR DESIGN PATENT APPLICATION (37 CFR 1.63) AND POWER OF ATTORNEY		Attorney Docket Number 2139-11US FC
		First Named Inventor Yasuo Konishi et al.
		<i>Complete if known</i>
		Application Number _____
		Filing Date _____
		Group Art Unit _____
		Examiner Name _____

Declaration Submitted with Initial Filing OR Declaration Submitted after Initial Filing (surcharge (37 CFR 1.16(e)) required)

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

TRIVALENT THROMBIN INHIBITOR

the specification of which

is attached hereto.
OR
 was filed on 10/15/1997
(mm/dd/yyyy)

as United States Application Number or PCT International Application Number
and was amended on _____ (if applicable).
(mm/dd/yyyy)

PCT/CA97/00745

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. 119(a)-(d) or 365(b) of any foreign application(s) for patent or inventor's certificate, or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or of any PCT international application having a filing date before that of the application on which priority is claimed.

Prior Foreign Application Number(s)	Country	Foreign Filing Date (MM/DD/YYYY)	Priority Not claimed	Certified Copy Attached?	
				YES	NO
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
			<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Additional foreign application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

I hereby claim the benefit under 35 U.S.C. 119(e) of any United States provisional application(s) listed below.

Application Number(s)	Filing Date (MM/DD/YYYY)	<input type="checkbox"/> Additional provisional application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto.

COMBINED DECLARATION FOR UTILITY OR DESIGN
PATENT APPLICATION (37 CFR 1.63) AND POWER OF ATTORNEY

PTO/SB/02A (3-97)

DECLARATION

ADDITIONAL INVENTOR(S)
Supplemental Sheet
Page 1 of 1

Name of Additional Joint Inventor, if any:

Given Name (first and middle [if any])

A petition has been filed for this unsigned inventor

Family Name or Surname

Jacek

SLON

Inventor's Signature

Residence:

City Montreal

State Quebec

Country CANADA

Date

6 Apr. 1/2000

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Citizenship Canadian

Apartment 301

City Montreal

Province
or State

Quebec

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H4P 2G6

Country CANADA

Name of Additional Joint Inventor, if any:

Given Name (first and middle [if any])

A petition has been filed for this unsigned inventor

Family Name or Surname

Inventor's Signature

Date

Residence:

City _____ State _____

Country _____

Citizenship _____

Post Office Address _____

City _____

Province
or State

Postal
Or Zip

Country _____

Name of Additional Joint Inventor, if any:

Given Name (first and middle [if any])

A petition has been filed for this unsigned inventor

Family Name or Surname

Inventor's Signature

Date

Residence:

City _____ State _____

Country _____

Citizenship _____

Post Office Address _____

City _____

Province
or State

Postal
Or Zip

Country _____

Additional inventors are being named on the

supplemental Additional Inventor(s) PTO/SB/02A attached hereto.

**COMBINED DECLARATION FOR UTILITY OR DESIGN
PATENT APPLICATION (37 CFR 1.63) AND POWER OF ATTORNEY**

I hereby claim the benefit under 35 U.S.C. 120 of any United States application(s), or 365(c) of any PCT international application designating the United States of America, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 CFR 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	Parent Patent Number (if applicable)

Additional U.S. or PCT International application numbers are listed on a supplemental priority data sheet PTO/SB/02B attached hereto:

As a named inventor, I hereby appoint the following registered practitioner(s) to prosecute this application and to transact all business in the Patent Trademark Office connected therewith:

Customer Number: **020988**



020988

PATENT AND TRADEMARK OFFICE

Direct all correspondence to:



020988

PATENT AND TRADEMARK OFFICE

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name of sole or First Inventor:

A petition has been filed for this unsigned inventor

Given Name (first and middle [if any])

Family Name or Surname

Yasuo

KONISHI

Inventor's Signature

Date

Apr. 16, 2000

Residence: City

Kirkland

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H9J 1K8

Country

CANADA

Additional inventors are being named on the

supplemental Additional Inventor(s) PTO/SB/02A attached hereto.